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## HOW LONG DOES A MOSQUITO RETAIN MALARIA PARASITES?

By BRUCE MAYNE, Associate Sanitarian, United States Public Health Service.

The following observations have been made recently in a preliminary study to determine the maximum length of time the plasmodium of malaria will remain viable in infected mosquitoes. In connection with this work, observations were made in regard to the longevity of anopheline mosquitoes. The determination of the average duration of infection in the mass of mosquitoes is of great sanitary importance, as is admitted by many writers on tropical parasitology.

It has an important application in the control of malaria by quinine sterilization of carriers, especially under tropical conditions. Also, quinine, to be an effective prophylactic, must be dispensed in immunizing doses as long as infected mosquitoes exist, else these mosquitoes may reinfect the human host once sterilized by quinine. Sanitary measures can not be safely discontinued in a region as long as infection persists in either host. It is important to know in connection with the control of malaria whether the time an *Anopheles* remains infective is materially less than its active life.

Accurate information relative to the longevity of the insect and the viability of the *Plasmodium* have an intimate bearing on the question of the latency of infection in the mosquito during hibernation. A study of the longevity of mosquitoes may also give us important information regarding the influence of food requirements and temperature on their activities.

Another interesting, if not important, sanitary application of the knowledge of duration of infection lies in the still unsolved question of the existence of an animal reservoir host for malaria. This question is constantly brought up by tropical travelers and explorers, in connection with the probability of acquiring malaria in localities where it is alleged no human being has set foot for a long period. It is reputed that some animal other than man is the host of the malarial fever indigenous to these tropical regions. This problem can not be considered intelligently unless it is known how

long mosquitoes harbor malaria parasites. If the longevity of the parasites in the mosquito under such conditions were known, it might be less difficult to account for the responsible host of the infection in supposedly uninhabited regions.

It must be appreciated that probably not more than 3 per cent of anophelines in the worst malarious districts of the United States are responsible for the majority, if not all, of the new infections of malaria that occur in those districts. This fact indicates the importance of a study of individual variations in the insect host in connection with gametocyte control.

The following data relative to the duration of life of the mosquito and its infectivity were obtained from preliminary studies conducted in the Public Health Service malaria laboratory at Memphis, Tenn., during 1920 and 1921.

#### LENGTH OF MOSQUITO LIFE.

The maximum length of time any anopheline was kept alive was 231 days. A laboratory-bred specimen of *A. punctipennis* was fed on dates and water exclusively and kept in a lantern globe at a relatively low temperature (45° to 75° F.) from May 24, 1920, to January 10, 1921. In one trial the average life of 85 specimens was found to be 90.4 days. Eight specimens lived from 175 to 203 days. In another trial 6 laboratory-bred specimens of *A. punctipennis* were allowed one feeding of blood and kept for the remainder of the time on water and the juice of dates. The temperature of the container was maintained, as before, between 45° and 75° F. These mosquitoes lived 176, 184, 186, 196, and 217 days, respectively.

In only one series were sufficient data obtained to give a comparison of the average life of the three species of mosquitoes common in this region. These specimens were given 1 to 3 feedings of blood, followed by a diet of fruit juices. The temperature of the containers was 48° to 76° F. The longevity was recorded as follows:

*A. punctipennis* (22 specimens)—100 days.

*A. crucians* (4 specimens)—65 days.

*A. quadrimaculatus* (6 specimens)—73 days.

A male specimen of *A. punctipennis* kept on fruit juices and water lived 89 days.

It is interesting to note that a culicine (*Culex territans*) kept under the same conditions as the other species mentioned lived a period of 265 days. It is stated that this particular species does not partake of mammalian blood, but sustains life on a vegetable diet, as, in this instance, fruit juices. It is supposed to thrive normally on the blood of frogs.

## DURATION OF INFECTION.

In this connection the writer has reported previously that a specimen of *Anopheles punctipennis* fed on a patient harboring *Plasmodium vivax* was found to retain a few scattered sporozoites, apparently degenerated, in two lobes of its salivary glands, for a period of 158 days. These organisms were presumably dead, lacking definite nucleus and devoid of motility.<sup>1</sup>

In the present series, 61 specimens of *A. punctipennis*, 8 specimens of *A. quadrimaculatus*, and 2 specimens of *A. crucians* were fed to engorgement on a patient in whose blood a moderate number of crescents were found. The mosquitoes were then kept at room temperature, 59° to 83° F., for six days, then placed in a cool chamber (temperature 44° to 78° F.), and given a diet of date juice and water for the remainder of the time. At the end of seven days a specimen of *A. punctipennis* was dissected; no parasites were found. Beginning on the thirty-fourth day after blood feeding, one, or more, of the mosquitoes was dissected daily. The longest period between feeding upon a human crescent carrier and dissection was 95 days. No signs of infection were observed in any of the specimens from this lot, with the exception of the glands of three mosquitoes (all *A. punctipennis*) in which were found disintegrated forms, probably dead sporozoites.

Several specimens of presumably infected *A. punctipennis* of another lot were applied to volunteers in order to test the viability of sporozoites possibly remaining in the glands. Four specimens of mosquitoes were induced to bite as late as 74 days after obtaining blood from a crescent carrier. No infections resulted from the inoculations during an observation period of 30 days. Upon dissection of these mosquitoes at various times, nucleated, sluggishly motile bodies, indistinguishable from sporozoites, were found in the glands of two of them. These two then retained living parasites of *P. falciparum* from 83 to 92 days.

In a similar test, an inoculation experiment on a human host proved successful 55 days after the infection of the mosquito. The new host proved to have clinically and microscopically a typical infection due to *P. falciparum*. The mosquito used in this experiment was dissected 13 days later, giving a maximum duration of infection of 68 days. Here typical, very active sporozoites were found.

A suggestion as to the possible range in duration of infectibility of anophelines is indicated in the following experiment:

<sup>1</sup> Mayne, Bruce: Can the Mosquito Convey Infection from a Malaria Patient Undergoing Treatment? Does Sporogony Affect Mosquito Life? Public Health Reports, vol. 35, No. 28, July 9, 1920, pp. 1664-1669. Reprint No. 602.

The same specimen of *A. punctipennis* that produced malaria after a period of 55 days was applied to another volunteer host on the sixty-seventh day and failed to communicate the infection. As noted above, this specimen was dissected on the sixty-eighth day and living sporozoites were found in its salivary glands. It had received one meal each from patient and volunteer host. In a similar test, under identical conditions, another specimen failed to convey the infection when applied to a volunteer host upon the sixty-first day, although this mosquito was found to harbor viable sporozoites when dissected 9 days later. The third specimen of this series harbored living sporozoites 71 days after obtaining gametocytes from the patient, although attempted transmission to a second host on the sixty-sixth day after infection failed.

During the present study, 95 days was the longest time that apparently dead sporozoites were found within the salivary glands of specimens of *A. punctipennis* infected with *P. falciparum*; and in those infected with *P. vivax*, 105 days was the maximum period.

#### TEST ADOPTED FOR DETERMINING VIABILITY OF SPOROZOITES.

The criteria for the presence of living sporozoites in the salivary glands of mosquitoes used in these experiments, other than that of inoculation into a human host, were as follows:

(a) *Motility*.—The well-recognized typical writhing movements with the occasional end to end motion of translation, sometimes followed by a gradual migration out of the field.

(b) *Staining*.—In the absence of characteristic motility, identification by means of Giemsa staining was relied on. Usually this means is ample to distinguish between viable and degenerated forms and to rule out extraneous objects, such as crystals, rod-shaped bacilli, and filiform artefacts generally.

(c) *Chemical test*.—A rough test, as suggested by Wenyon, for the elimination of sporozoitelike bodies which may be confusing to the worker, is suspension of suspected material for several hours on a glass slide in media such as physiological salt solution, distilled water, and dilute acids. Artefacts disappear under this treatment.

#### SUMMARY.

1. The longest period of survival of uninfected *Anopheles* kept under artificial conditions on a diet of split dates and water, at a temperature of 45° to 75° F., was 231 days. A lot of 85 specimens of *A. punctipennis* kept without blood lived an average of 90.4 days. Eight of these were kept a period of 175 to 203 days. In mosquitoes of this species, given 1 to 3 feedings of blood previous to a diet of fruit juices, 22 specimens averaged a longevity of 100

days, and 6 specimens lived 176 to 217 days. A single specimen of *Culex territans* survived 265 days on a diet exclusively of fruit juices at a temperature of 48° to 76° F.

2. Plasmodia of malaria distinctly recognizable by their morphology and staining were detected in the salivary glands of five specimens of *A. punctipennis*, 68, 70, 71, 83, and 92 days, respectively, after infection. These mosquitoes had been allowed to bite a crescent carrier on a single occasion and were maintained at room temperature (59° to 83° F.) for 6 days, then kept in a container registering temperatures of 44° to 78° F. for the remainder of the experiment.

3. Plasmodia of malaria proved to be viable by inoculation into a human host from the bite of a mosquito infected 55 days previously. Mosquitoes failed to convey malaria plasmodia through their biting, 61, 66, and 67 days, respectively, after becoming infected (gland sporozoites obtained). These three mosquitoes were kept under conditions identical with those in which viable sporozoites were demonstrated in the five specimens mentioned above.

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## ON THE PROBABLE IDENTITY OF THE CHITTENDEN-UNDERHILL PELLAGRALIKE SYNDROME IN DOGS AND "BLACK-TONGUE."

### WITH REPORT OF NECROPSY FINDINGS IN TWO CASES OF BLACK-TONGUE.

By G. A. WHEELER, Passed Assistant Surgeon, and JOSEPH GOLDBERGER, Surgeon, United States Public Health Service; and M. R. BLACKSTOCK, D. V. S., Spartanburg, S. C.

We desire to invite attention to the striking similarity and probable identity of Chittenden and Underhill's pellagralike syndrome in dogs and the condition known to American veterinarians as "black-tongue."<sup>1</sup>

In August, 1917, Chittenden and Underhill reported the production in dogs of a pathological condition which they regarded as closely resembling human pellagra. The condition was described as follows:

"The onset of the pathological symptoms is generally very sudden. Usually the first abnormal manifestation is a refusal to eat, and examination will reveal nothing to account for the loss of appetite. The animal lies quietly in its pen and is apathetic. After continued refusal to eat for a day or two, the mouth of the dog will present a peculiar and characteristic appearance. The inner surface of the cheeks and lips and the edges of the tongue are so covered with pustules as to give the impression of a mass of rotten flesh. The odor from these tissues is foul and almost unbearable. When stroked with absorbent cotton the mucous lining of the mouth comes

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<sup>1</sup> Synonyms: Sore mouth, southern canine plague, dog typhus, dog typhoid, gastroenteritis hemorrhagica, Stuttgart dog epizootic.